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Titolo abstract

Bioequivalence assessment from crossover data: a new approach for the construction of confidence intervals for the ratio of two formulation means

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Bioequivalence assessment from crossover data: a new approach for the construction of confidence intervals for the ratio of two formulation means

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Introduction

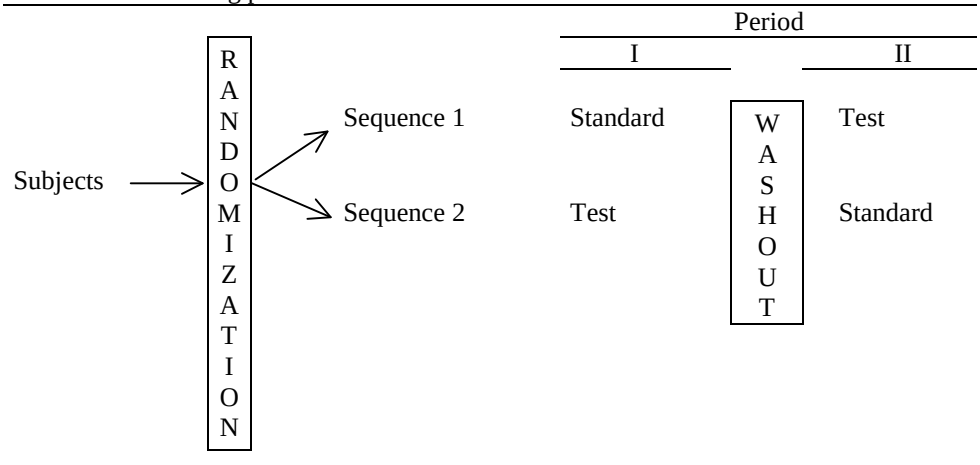
The assessment of bioequivalence for different drug formulations is based on the following fundamental bioequivalence assumption: "When two drug formulations are equivalent in the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed and becomes available at the site of drug action, it is assumed that they will be therapeutically equivalent". The purpose of bioequivalence trials is to identify pharmaceutical equivalents or pharmaceutical alternatives that are intended to be used interchangeably for the same therapeutic effects. Thus, bioequivalent drug formulations are therapeutic equivalents and can be used interchangeably (Chow and Liu, 2000).

Because the response of individual subjects participating in the study may differ considerably, it is recommendable to remove the inter-subject variability from the comparison between formulations. Thus, a two-period crossover design (Table 1) on univariate characteristics of rate and extent (for example, area under the concentration curve, AUC and maximum concentration, C_{max}) is usually used. To claim bioequivalence in average bioavailability, the ±20 rule requires that the ratio of the mean of the test formulation (T) and the mean of the standard formulation (S), called $R = \mu_T / \mu_S$, for AUC and C_{max} be within (80%, 120%) limits (Berger and Hsu, 1996). In the last two decades, several statistical methods based on untransformated data have been proposed, including the confidence intervals (CIs) approach, the method of interval hypotheses testing, the Bayesian approach and nonparametric methods. For the parametric CIs approach, several authors proposed the application of the Fieller's theorem to construct a CI for μ_T / μ_S and compare it with (80%, 120%) limits (Mandallaz and Mau, 1981; Locke, 1984; Liu, 1990; Hsu, Hwang et al., 1994; Vuorinen and Tuominen, 1994). However, the Fieller method (FM) does not always exist, i.e. the CIs are unbounded for the ratio of the two formulation means. We propose a new parametric technique for the construction of 100(1-α)% CIs, based on the exact distribution of the two estimated formulation means.

Material and methods

Suppose that in "Sequence 1" the standard formulation is given first and the test formulation is given second. In "Sequence 2", the formulations are given in the reverse order, as reported in Table 1.

Table 1 – Two-period crossover design for comparing a test formulation and a standard formulation of a drug product.



Let X_{ijS} and X_{ijT} be the responses (e.g., AUC and/or C_{max}) of the j^{th} subject (with $j=1,\ldots,n$) in the i^{th} sequence (with $i=1,2$) for the standard formulation and the test formulation, respectively. For simplicity, assume that each sequence has the same number of subjects n. However, this restriction can be removed and the statistical methodologies can be easily extended to the more general setting (Locke, 1990; Chow and Liu, 2000). The model has these following assumptions. The subjects are considered to be a random sample from a large population. For each sequence, X_{ijS} and X_{ijT} have a Bivariate Normal (BN) distribution. It is assumed that the covariance matrix of X_{ijS} and X_{ijT} is the same for both sequences. In addition to the effects of the

formulations, the means of X_{ijS} and X_{ijT} are affected by period effects. Sequence effects are also included in the model. The four means for the model are the following:

$$E(X_{1jS}) = \mu_S + \pi_1 + \alpha_1 \quad \text{and} \quad E(X_{1jT}) = \mu_T + \pi_2 + \alpha_1$$
$$E(X_{2jS}) = \mu_S + \pi_2 + \alpha_2 \quad \text{and} \quad E(X_{2jT}) = \mu_T + \pi_1 + \alpha_2$$

where μ_S and μ_T are the population means for the standard and test formulations, π_1 and π_2 are the period effects, and α_1 and α_2 are the sequence effects. The constraints $\pi_1 + \pi_2 = 0$ and $\alpha_1 + \alpha_2 = 0$ are assumed.
The parameter of interest is defined as the ratio of the two formulation means, called $R = \mu_T / \mu_S$, with $\mu_S \neq 0$. By means of data from a comparative bioavailability study, we obtain the maximum likelihood estimators (MLE) for the means $\underline{\hat{\mu}} = (\hat{\mu}_T, \hat{\mu}_S)$ and covariance matrix $\hat{\Sigma}$, as follows:

$$\hat{\mu}_T = \bar{X}_{..T} = \frac{1}{2n}\sum_{i=1}^{2}\sum_{j=1}^{n} x_{ijT} \quad \text{and} \quad \hat{\mu}_S = \bar{X}_{..S} = \frac{1}{2n}\sum_{i=1}^{2}\sum_{j=1}^{n} x_{ijS}$$
$$\hat{\sigma}_T^2 = \frac{1}{2n-2}\sum_{i=1}^{2}\sum_{j=1}^{n}\left(x_{ijT} - \bar{x}_{i.T}\right)^2 \quad \text{and} \quad \hat{\sigma}_S^2 = \frac{1}{2n-2}\sum_{i=1}^{2}\sum_{j=1}^{n}\left(x_{ijS} - \bar{x}_{i.S}\right)^2$$
$$\hat{\sigma}_{ST} = \hat{\sigma}_{TS} = \frac{1}{2n-2}\sum_{i=1}^{2}\sum_{j=1}^{n}\left(x_{ijT} - \bar{x}_{i.T}\right)\left(x_{ijS} - \bar{x}_{i.S}\right).$$

These MLEs are consistent for the true values and by the invariance property of the MLEs the consistent MLE of *R* is $\hat{R} = \hat{\mu}_T / \hat{\mu}_S$ (Stuart, Ord et al., 1999).
The aim is to construct a $100(1-\alpha)\%$ CIs for *R* with the FM and the new method and to provide comparisons between the two methods.

The Fieller Method (FM)
The FM refers to a general approach to obtain CIs for the ratio of means in a BN random variable (rv) (Fieller, 1954). The FM assumes that numerator and denominator of the ratio estimator $\hat{R} = \hat{\mu}_T / \hat{\mu}_S$ follow a BN distribution, so that $\hat{\mu}_T - R\hat{\mu}_S$ is normally distributed with expected value equal to zero. By means of the standardization of $\hat{\mu}_T - R\hat{\mu}_S$, Fieller found a pivotal quantity for the unknown parameter *R*, called *Q* as follows:

$$Q = \frac{\hat{\mu}_T - R\hat{\mu}_S}{\sqrt{\left(\hat{\sigma}_T^2 - 2R\hat{\sigma}_{ST} + R^2\hat{\sigma}_S^2\right)/(2n)}} \sim Student(2n-2).$$

Therefore, the CIs for *R*, if they exist, are derived from the following inequality: $\left(\hat{\mu}_T - R\hat{\mu}_S\right)^2 \leq t^2_{(1-\alpha/2)} Var\left(\hat{\mu}_T - R\hat{\mu}_S\right)$ where $t_{(1-\alpha/2)}$ is the (1-α/2)th quantile point of a Student rv with (2n-2) degrees of freedom. The second order inequality may be conveniently expressed as $f(R) = a_n R^2 - 2b_n R + c_n \leq 0$ with $a_n = \hat{\mu}_S{}^2 - (1/(2n))t^2_{(1-\alpha/2)}\hat{\sigma}_S^2$, $b_n = (1/(2n))t^2_{(1-\alpha/2)}\hat{\sigma}_{ST} - \hat{\mu}_T\hat{\mu}_S$, $c_n = \hat{\mu}_T^2 - (1/(2n))t^2_{(1-\alpha/2)}\hat{\sigma}_T^2$. The CIs for *R* are bounded only when $a_n > 0$, i.e. the estimated mean of the standard formulation $\hat{\mu}_S$ is significantly different from zero at level α (Locke, 1984; Liu, 1990; Hsu, Hwang et al., 1994; Vuorinen and Tuominen, 1994). When this condition is verified, the lower limit ($\hat{R}_L$) and the upper limit ($\hat{R}_U$) of the CI for *R* are:

$$\hat{R}_L = \frac{-b_n - \sqrt{b_n^2 - a_n c_n}}{a_n} \quad \text{and} \quad \hat{R}_U = \frac{-b_n + \sqrt{b_n^2 - a_n c_n}}{a_n}.$$

The Exact Distribution Method (EDM)
On the same parametric assumption of FM, the distribution of $\underline{\hat{\mu}} = (\hat{\mu}_T, \hat{\mu}_S)$ is a BN rv with means $\underline{\mu} = (\mu_T, \mu_S)$, variances $\left(\hat{\sigma}_T^2/2n; \hat{\sigma}_S^2/2n\right)$ and coefficient of correlation $\hat{\rho} = \hat{\sigma}_{ST} / \sqrt{\hat{\sigma}_S^2\hat{\sigma}_T^2}$. Therefore, $\hat{R} = \hat{\mu}_T / \hat{\mu}_S$ is the ratio of two correlated Normal rvs jointly distributed as a BN rv, and its distribution is a finite non-standard mixture density with dichotomous proportions with a Cauchy component (Marsaglia, 2006; Galeone, 2007). The simultaneous CIs for $\hat{R} = \hat{\mu}_T / \hat{\mu}_S$ can be obtained by using the inverse cumulative density function of $\hat{R}$, as follows:

$$\Pr\left\{\hat{R}_{\frac{\alpha}{2}} = \hat{R}_L < R < \hat{R}_U = \hat{R}_{\left(1-\frac{\alpha}{2}\right)}\right\} = (1-\alpha)$$

where $\hat{R}_{\frac{\alpha}{2}} = F_{\hat{R}}^{-1}\left(\frac{\alpha}{2}\right)$ is the $(\alpha/2)^{th}$ quantile point and $\hat{R}_{1-\frac{\alpha}{2}} = F_{\hat{R}}^{-1}\left(1-\frac{\alpha}{2}\right)$ is the $(1-\alpha/2)^{th}$ quantile point of the distribution of $\hat{R}$. This method warrants the existence of the CIs, since the cumulative density function (CDF) is a monotonic non-decreasing function that can always be inverted.

Simulation study

Monte Carlo experiment was used to assess the performances of the FM and EDM for computing 90% CIs for *R*, by differing levels of correlation between numerator and denominator. We started using a simulated population with known means (0.25, 1.20) and variances (9, 16) of the two formulations, respectively, known correlations between test and standard formulations (0, |0.3|, |0.6|, |0.9|) and a known *R*. The sample size varied from 25 to 1,600 with the rule of the doubling technique. Overall, there were 49 combinations of simulation parameters. For each combination of parameters, we simulated 5,000 independent samples for each treatment group from this population. The criterions used to evaluate the performances of the methods were the probability of coverage of the intervals (denoted as $(1-\hat{\alpha})$), the average width of the intervals (denoted as *Amp*) and the symmetric miscoverage of the intervals (denoted as *%ds*).

Results

The performances of the two methods for the construction of 90% CIs for *R*, for $\rho = 0.6$, were reported in Table 2. For small values of *n* ($n \leq 200$) there was at least one unbounded CI that yielded the average widths not to be expressed as a real number. Consequently, the corresponding coverage probabilities were very low. For elevated values of *n*, the performances of the CIs based on FM and EDM were very close.

Table 2 – Performances of the two methods for the construction of 90% CI with $\rho = 0.6$.

		FM	EDM
n			
25	$(1-\hat{\alpha})$	0.1999	0.9169
	%ds	0.8376	0.5321
	Amp	-	4.2393
50	$(1-\hat{\alpha})$	0.3164	0.9104
	%ds	0.8266	0.6004
	Amp	-	2.8690
100	$(1-\hat{\alpha})$	0.8224	0.9064
	%ds	0.7635	0.6106
	Amp	-	1.5660
200	$(1-\hat{\alpha})$	0.8703	0.9004
	%ds	0.6810	0.4960
	Amp	-	0.6908
400	$(1-\hat{\alpha})$	0.8960	0.8996
	%ds	0.5192	0.5180
	Amp	0.3953	0.3442
800	$(1-\hat{\alpha})$	0.9028	0.9028
	%ds	0.5374	0.5342
	Amp	0.2631	0.2628
1600	$(1-\hat{\alpha})$	0.9032	0.9029
	%ds	0.5353	0.5372
	Amp	0.1817	0.1816

Extending the simulation results to all other values of ρ considered, the FM always failed for $n \leq 50$, with corresponding non-acceptable coverage probabilities. For ρ equal to -0.6 and -0.9, the FM failed also for *n* equal to 100, but in these cases the coverage probabilities were higher as referred to those for $n<100$. For other values of ρ, i.e. equal to –0.3, 0 and 0.3, the FM failed also for *n* equal to 200. The simulation results highlighted that the FM less frequently produces unbounded confidence intervals for *R* with increasing values of *n*. Finally, the performances of the two methods were satisfactory and very close to each other for high values of *n*.

Conclusions

The EDM for the construction of CIs for $\hat{R} = \hat{\mu}_T / \hat{\mu}_S$ always exists and produces bounded intervals with satisfactory and very close performances to the FM. Although the calculus of the limits of the CIs by means of the new method is more complicated, as this involves the calculation of the inverse of a CDF that can be obtained only by a computer support, the EDM always allows to obtain bounded CIs, also when the FM produces unbounded intervals. The implementation of procedures and functions to construct CIs with the EDM is already available in Matlab and will soon be available in SAS package, too. Differently from other parametric methods for the construction of CIs for $\hat{R} = \hat{\mu}_T / \hat{\mu}_S$, these two methods are preferable because they take into account not only the variability of $\hat{\mu}_S$ but also the intersubject variability. Finally, the EDM is easily extended to the general crossover designs, as was the FM proposed by Locke (1990).

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